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The risk assessment of *Vibrio parahaemolyticus* in raw oysters in Taiwan under the seasonal variations, time horizons, and climate scenarios

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ABSTRACT

While seafood is consumed worldwide, eating raw seafood is more popular in Asian countries. Consuming seafood promotes many health benefits, but is not risk free because it can be contaminated with foodborne pathogens, which can result in disease outbreaks. In this study, we performed the risk assessment of *Vibrio parahaemolyticus* in raw oysters in Taiwan under the seasonal variations, time horizons, and climate scenarios. Sixty-four scenarios with 100,000 iterations were performed with Monte Carlo simulation. The results showed the estimated risk of eating oysters under the baseline to be 2.2×10^{-5}, 4.5×10^{-5}, 9.4×10^{-5}, and 6.2×10^{-5} per serving in winter, spring, summer, and fall, respectively. The risk in that baseline in this study was found to be intensified by 23%, 35%, 37%, and 65% by RCP2.6, RCP4.5, RCP6.0, and RCP8.5, respectively, and it was intensified to 0.18, 0.38, and 0.64 times in 2016–2035, 2046–2065, and 2081–2100, respectively. Mitigation strategies simulated in this study show that the risk in that baseline could be reduced by 64–87%, 52–67%, and 47–63% with immediate refrigeration, depuration, and freezing treatment, respectively, and could be reduced by up to 100% by mild thermal treatment, thermal shock and irradiation. Reducing and or controlling the risk of raw oysters will prevent foodborne outbreaks from occurring. Therefore, the findings obtained from this study may help the food safety authority and food managers in food industry in their decision-making process.

1. Introduction

The issue of climate change and its variability has become a global concern. The Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC) assessed that global climate change may affect the weather, which can result in changes in the air and seawater temperatures, ocean acidity, sea level, weather disturbances, freshwater cycle, and rainfall and wind patterns (IPCC, 2014). The impact of climate changes is also expected to worsen over the next decades. More specifically, the global temperature may possibly increase up to 2.6 °C in 2046–2065 and 4.8 °C in 2081–2100. These changes may not only affect the biodiversity, but also the quality and safety of food, which can pose a significant threat to human health (Marques et al., 2010; Tirado et al., 2010). Towards this, the European Food Safety Authority (EFSA) recently highlighted that increasing seawater temperatures could be a potential emerging food safety issue because it may affect the growth of foodborne pathogens, especially *Vibrio* spp. (EFSA, 2018).

Several studies have reported that climatological variables substantially influenced the presence and number of certain *Vibrio* spp. in marine environment and seafood (Johnson et al., 2012; Parveen et al., 2008; Reyes-Velázquez et al., 2010; Robles et al., 2013; Takemura et al., 2014; Tey et al., 2015; Yu et al., 2013). Seawater temperature is among other variables that mostly associated with the population of *Vibrio* spp. in seafood (Curriero et al., 2017; Johnson et al., 2012; Malayil et al., 2013; Parveen et al., 2008; Reyes-Velázquez et al., 2010; Robles et al., 2013; Tey et al., 2015). Other studies, however, have observed that seawater temperature was not a significant factor (Deepanjali, Kumar, Karunasagar, & Karunasagar, 2005; Malayil et al., 2013; Moore et al., 2014; Ramos et al., 2014; Yu et al., 2013). Seawater salinity is another factor that was also often associated with the densities of *Vibrio* spp. in seafood (Blackwell & Oliver, 2008; Hsieh, Fries, & Noble, 2007; Tey et al., 2015; Yu et al., 2013; Zimmerman et al., 2007). In other studies, the population of *Vibrio* spp. in seafood and marine environment also correlated with plankton, chlorophyll-a, turbidity, and dissolved oxygen (Kirschner et al., 2011; Oberbeckmann et al., 2012; Tey et al., 2015). A recent systematic literature synthesis performed by Takemura et al. (2014) reported that these climatological variables have differing effects on the population of *Vibrio* spp.

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depending on species, geographic location or environmental niche.

Vibrio parahaemolyticus is one of the major seafood-borne gastroenteritis causing bacteria which frequently isolated from seafood samples. It is important to note that not all strains of *V. parahaemolyticus* are pathogenic; only the member of this pathogen which had virulence traits of thermostable direct hemolysin (Tdh) and Tdh-related hemolysin (Trh), encoded by *tdh* and *trh*, respectively, is considered pathogenic (Raghunath, 2015). This pathogen was the main cause of foodborne outbreaks originating from contaminated seafood in Taiwan. Cheng et al. (2013) documented that 28.8% of the 4,282 foodborne outbreaks between 1991 and 2010 were caused by *V. parahaemolyticus*. A later report by Lin et al. (2015) documented that 18% of the 7,126 foodborne outbreaks in Southern Taiwan between 2004 and 2013 were caused by eating food contaminated by *V. parahaemolyticus*. Collected data from the Taiwan Food and Drug Administration (TFDA) between 2000 and 2011 (as cited in Hsiao, Jan, & Chi, 2016) showed that 751 out of 3,485 foodborne outbreaks in Taiwan were caused by this pathogen.

A growing body of literature evaluated the food safety risk of seafood in regard to health impact assessment, best-management practices, and qualitative risk matrices (Hamilton et al., 2018; Huang, Hwang, Huang, Wu, & Hsiao, 2018; Sani et al., 2013). However, very few studies have included and examined the impact of climate change on microbial food safety in seafood (Ortiz-Jiménez, 2018; Sobrinho et al., 2014). Including this factor is necessary to allow the estimation of its future impacts on seafood safety, which could be used as early warning information (Lenton, 2011). Not to mention, extreme climate change probably occurs because there was still no agreement among the members to decrease the greenhouse effect (IPCC, 2014). The impact of climate changes has been recognized by the Taiwanese government under the Adaptation Strategy to Climate Change in Taiwan (National Development Council of Taiwan, 2012). This case study, therefore, was performed to provide baseline information in an effort to control and/or reduce the impact of climate change on food safety. Mitigation strategies were also simulated in this study to inform risk management practices of oyster chains in Taiwan. Quantitative microbial risk assessment (QMRA) is considered as an adequate approach to bridge this gap because it enables the identification and evaluation of the best intervention to reduce food safety risk in a food product. The QMRA of *V. parahaemolyticus* in this study was performed to evaluate the impact of climate changes in different seasons and time horizons.

2. Methodology

2.1. Distribution of air and seawater temperature

Table 1 shows the mean 8-d seawater surface temperature (T_w) from 2013 to 2017 according to the season in Taiwan. Seasons were defined by calendar month as follows: winter (December–February), spring (March–May), summer (June–August) and fall (September–November). The T_w was obtained from radiance measurements were collected by the Moderate Resolution Imaging Spectroradiometer (MODIS) instruments aboard NASA's Terra and Aqua satellites computed by the National Aeronautics and Space Administration (NASA).[1] The T_w estimates are available at a spatial resolution of 1 km and based on composite 8-d values computed with the accuracy of half a degree Celsius. We used the coordinates of 18°3′0″N – 26°3′0″N, 117°2′60″E – 123°57′0″E. The distribution of seawater was then obtained by sampling data generated by the bivariate normal distribution. The value of mean (μ), standard deviation (σ) and correlation (ρ) were estimated by using the method of moments (Montgomery & Runger, 2010).

[1] https://neo.sci.gsfc.nasa.gov/view.php?datasetId=MYD28M.

Table 1
Seasonal distribution of mean daily 8-d seawater temperature (T_w) in Taiwan, between 2013 and 2017.

Season	μ_μ	σ_μ	μ_σ	σ_σ	ρ
Winter	23.19	0.56	2.21	0.25	−0.71
Spring	25.83	1.70	1.97	0.22	−0.88
Summer	29.34	0.28	0.61	0.37	−0.62
Fall	27.32	1.20	0.93	0.30	−0.92

Notes: μ_μ is the mean temperatures, σ_μ is the standard deviations of the temperatures, μ_σ is the means of the standard deviations of daily temperatures, σ_σ is the standard deviations of the standard deviations of temperatures, and ρ is the correlation coefficients of temperatures and standard deviations of temperatures.

2.2. Simulation of climate scenarios

Climate scenarios were developed based on the defined future scenarios of greenhouse gas emissions denominated in Representative Concentration Pathways (RCPs) suggested by the IPCC. Table 2 shows the distribution of temperature increase projected in the IPCC (2013) report, Annex II, p. 1444. These scenarios were RCP2.6 to represent a stringent mitigation scenario, RCP4.5 and RCP6.0 to represent the intermediate scenarios, and RCP8.5 to represent a very high level of greenhouse gas emissions (IPCC, 2013). These RCPs were then simulated in this study to evaluate their impact on the risk of consuming raw oysters in Taiwan.

2.3. Simulation model: case study of raw oyster consumption

In this study, the risk of consuming oysters was assumed as the effect of infection of pathogenic *V. parahaemolyticus*. The infection caused by this organism can cause human gastroenteritis, depending on the number of these bacteria in oysters that may be consumed. The number of *V. parahaemolyticus* in water and oyster was estimated according to a function of seawater temperature (T_{sea}) and salinity (Sal). The growth of *V. parahaemolyticus* from harvest to consumer was estimated according to a function of time and temperature.

2.4. Mitigation strategies

Six mitigation strategies were simulated in this study (Table 3), which focused on reducing the number of *V. parahaemolyticus* in oysters. These strategies were immediate refrigeration, immersion in seawater with ultraviolet (UV) light, frozen storage at −20 °C for 35 d, thermal treatment at 50 °C for 5 min, and thermal shock at 50 °C for 5 min followed by a rapid cooling, and irradiation. These strategies were previously suggested by the US Food and Drug Administration (US FDA, 2005) and Ortiz-Jiménez (2018) to eliminate/reduce the number of this pathogen in shellfish. Besides, simulating mild thermal treatment, thermal shock, or irradiation treatment are necessary because these treatments have not been implemented by shellfish producers nor suggested by the food safety authority in Taiwan (TFDA, 2018). Details of the assumption used in mitigation strategies are given in Appendix 1 to conserve space.

2.5. Quantification of food safety risk of consuming oyster

The exposure modeling was performed based on the general raw oysters' chains in Taiwan. The QMRA model developed by Huang et al. (2018) with several modifications was used in this study. Improvement was made to this model to incorporate the impact of climate change variability on the risk of consuming raw oyster in Taiwan. The exposure pathways of oyster before eating were the culturing environment, post-harvest, and the exposure to the environment during transport.

The density of *V. parahaemolyticus* at harvest (Vp_h) as affected by the

Table 2
Temperature distribution based on the Representative Concentration Pathways (RCPs).

Season	RCP2.6	RCP4.5	RCP6.0	RCP8.5
Baseline	0	0	0	0
2016–2035	*RiskPert*(0.42, 0.65, 1.2)	*RiskPert*(0.48, 0.7, 1.0)	*RiskPert*(0.4, 0.6, 1.0)	*RiskPert*(0.5, 0.8, 1.2)
2046–2065	*RiskPert*(0.40, 1.0, 1.6)	*RiskPert*(0.9, 1.4, 2.0)	*RiskPert*(0.8, 1.3, 1.8)	*RiskPert*(1.4, 2.0, 2.6)
2081–2100	*RiskPert*(0.3, 1.0, 1.70)	*RiskPert*(1.1, 1.8, 2.6)	*RiskPert*(1.4, 2.2, 3.1)	*RiskPert*(2.6, 3.7, 4.8)

Source: The forth report of IPCC (2014).

Table 3
Mitigation strategies to control and/or reduce the risk of eating raw oysters.

Mitigation strategies[a]		Variables
M_0	No mitigation	0
M_1	Immediate refrigeration	The temperature during cleaning and packaging was truncated to 7 °C
M_2	Depuration	*RiskUniform*(0, 2.4)
M_3	Freezing and storage	*RiskUniform*(0, 2)
M_4	Mild thermal treatment	*RiskUniform*(4.5, 6)
M_5	Thermal shock	*RiskUniform*(5, 7)
M_6	Irradiation	6

[a] Mitigation strategies were explained in section *2.4* and Appendix 1.

seawater temperature and salinity was calculated based on the Tobit parameters suggested by the US FDA (2005) in Eq. (1) because of unavailability model in Taiwan. This model was developed based on a large survey of *V. parahaemolyticus* densities in oyster samples collected from growing areas on the Atlantic and Gulf coasts in the United States.

$$Vp_h = \alpha + \beta T_w + \gamma_1 Sal + \gamma_2 Sal^2 + RiskNormal(0,\sigma) \quad (1)$$

The parameters α and β are the regression parameters for the temperature effect, γ_1 and γ_2 are parameters for the salinity effect, and σ is the variance of combined effect. The maximum likelihood estimates for each parameter of this model was set as follow: $\alpha \sim$ *RiskPert*(-2.76, −2.05, −1.34), $\beta \sim$ *RiskPert*(0.087, 0.097, 0.11), $\gamma_1 \sim$ *RiskPert*(0.13, 0.2, 0.27), $\gamma_2 \sim$ *RiskPert*(-0.0073, −0.0055, −0.0038), and $\sigma \sim$ *RiskPert*(0.68, 0.73, 0.79) (US FDA, 2005). By using this model, seawater temperature and salinity were considered as significant factors. The seawater salinity of oysters culturing environments in Taiwan as reported by Yu et al. (2013) was assumed as a parameter of pert function with minimum, most likely and maximum of 20.6, 30.52, and 33.6, respectively. Furthermore, the level of *V. parahaemolyticus* during cleaning and packaging was considered as being affected by exposure to ambient temperature and time. The effect of temperature on the growth rate of *V. parahaemolyticus* in oyster during cleaning and packaging (μ_{proc}) was calculated based on the model developed by the US FDA (2005), whereas the inactivation rate was obtained from Huang et al. (2018) as shown in Eqs. (2)–(4); they reported that the oysters were cleaned and packed prior to distribution. The exposure time during cleaning and packaging was assumed as a variable of uncertainty modeled as a pert distribution with minimum, most likely, and maximum value of 5 h, 7 h, and 10 h, respectively (Huang et al., 2018). Thus, the maximum level of *V. parahaemolyticus* during cleaning and packaging (Vp_{proc}) was estimated in Eq. (5). The effect of immediate refrigeration (M_1) or depuration (M_2) on the level of *V. parahaemolyticus* in oysters was estimated in Eq. (6).

$$\mu_{proc} = IF(T_{air} < 12, \mu_{inactivation}, \mu_{\max},) \quad (2)$$

$$\mu_{inactivation} = 30.368\left[\frac{1}{T_{air} + 273.15}\right] - 0.1007 \quad (3)$$

$$\mu_{\max} = \left\{0.0112\left[\frac{9}{5}T_{air} + 32\right] - 0.4689\right\}^2 \quad (4)$$

$$Vp_{proc} = Vp_h + \mu_{proc} \times t_{proc} \quad (5)$$

$$Vp_{m_1} = Vp_{proc} - M_{1,2} \quad (6)$$

Next, the delivery time of oysters from harvest to restaurant (t_{trans}) in Taiwan was reported as a pert distribution parameter with a minimum, most likely, and a maximum value of 4 h, 24 h and 32 h (Huang et al., 2018). Huang et al. (2018) also noted that the distribution of temperature during transport (T_{trans}) was a parameter of logistic function with $\alpha = 7.0$ and $\beta = 3.2$. The growth or inactivation of *V. parahaemolyticus* as affected by the temperatures during transport was estimated in Eqs. (7)–(9). Hence, the maximum level of pathogenic *V. parahaemolyticus* after transport (Vp_{trans}) was estimated in Eq. (10). The effect of freezing (M_3), mild thermal treatment (M_4), thermal shock (M_5), or irradiation (M_6) on the level of *V. parahaemolyticus* in oysters was estimated in Eq. (11).

$$\mu_{trans} = IF(T_{trans} < 12, \mu_{inactivation}, \mu_{\max},) \quad (7)$$

$$\mu_{inactivation} = 30.368\left[\frac{1}{T_{trans} + 273.15}\right] - 0.1007 \quad (8)$$

$$\mu_{\max} = \left\{0.0112\left[\frac{9}{5}T_{trans} + 32\right] - 0.4689\right\}^2 \quad (9)$$

$$Vp_{trans} = Vp_{proc} + \mu_{trans} \times t_{trans} \quad (10)$$

$$Vp_{m_2} = Vp_{trans} - M_{3,4,5,6} \quad (11)$$

On the basis of the most recent available data, the characteristics of oyster consumption in Taiwan reported by Huang et al. (2018) were used in this study. According to Huang et al. (2018), the average weight of a single oyster (W) was described by the lognormal relationship with minimum and standard deviation value of 6.39 g and 2.4 g, respectively. The number of oysters in a serving was reported as a parameter of triangle function with minimum, most likely, and maximum value of 4, 6, and 10, respectively (Huang et al., 2018). Thus, the serving size of oysters (S) was estimated as a product of W and the number of oysters in a serving. The final number of total *V. parahaemolyticus* that may be consumed by Taiwanese in a serving of oysters (Vp_{serv}) was estimated by multiplying the serving size of oysters (S) and the estimated level of this organism after treatment (Vp_{m_2}).

The probability of acquiring infection caused by *V. parahaemolyticus* (P_{ill}) was estimated by the dose-response of Beta-Poisson (Huang et al., 2018; US FDA, 2005), as shown in Eq. (12).

$$P_{ill} = 1 - \left(1 + \frac{Vp_{cons}}{\beta}\right)^{-\alpha} \quad (12)$$

where α and β are the likelihood parameters of the function (US FDA, 2005).

Yu et al. (2013) reported that 367 out of 2623 oyster samples harvested from culturing environment in Taiwan contained *V. parahaemolyticus* but only 3 out 1073 oyster samples showed a characteristic of putative pathogenic strains. Thus, a Bayesian approach was used to describe the proportion of oyster samples being contaminated with *V. parahaemolyticus* (P_{Vp}) and the fraction of pathogenic strain of this microorganism which had virulence factor (*trh/tdh*) (P_{path}). The Beta distribution (s + 1, n − s + 1) was then used to estimate P_{Vp} with s = 364 and n = 2624. The same function, Beta-distribution, was used to estimate P_{path} with s = 4 and n = 1074. Finally, the risk per serving of

Table 4
Exposure assessment of Vibrio parahaemolyticus in oysters affected by the climate scenarios, seasonal variations, and time-horizons.

Symbol	Description	Variables	Units
	Post-harvest		
T_w	Sea water temperature	Table 1	°C
Sal	Salinity	*RiskPert*(20.6, 30.52, 33.6)	ppt
Vp_h	Level of Vp in oyster at harvest	Eq. (1)	log cfu/g
	Cleaning and packaging		
t_{proc}	Time spent in cleaning and packaging	*RiskPert*(5, 7, 10)	h
T_{proc}	Temperatures during cleaning and packaging	Table 1	°C
μ_{proc}	Growth rates during cleaning and packaging	Eqs. (3)–(5)	h^{-1}
Vp_{proc}	Maximum level of pathogenic Vp after cleaning and packaging	Eq. (6)	log cfu/g
Vp_{m1}	Maximum level of pathogenic Vp after depuration or refrigeration	Eq. (7)	log cfu/g
	Transport		
t_{trans}	Time spent in transport	*RiskPert*(4, 24, 32)	h
T_{trans}	Temperature in transport	*RiskLogistic*(7.044, 3.198)	°C
μ_{trans}	Growth or survival	Eqs. (8)–(10)	h^{-1}
Vp_{trans}	Maximum level of pathogenic Vp after transport	Eq. (11)	log cfu/g
Vp_{m2}	Maximum level of pathogenic Vp after treatment	Eq. (12)	log cfu/g
	Consumption		
W	Weight of an oyster	*RiskLogNormal*(6.89, 2.38)	g
S	Serving size	*RiskTriang*(4 × *W*, 6 × *W*, 10 × *W*)	g
Vp_{serv}	Maximum level of pathogenic Vp per serving	$10^{Vpm} \times S$	cfu/serving
	Risk characterization		
P_{ill}	Probability of illness of eating oyster per serving	Eq. (13)	per serving
P_{path}	Fraction of pathogenic Vp in oysters	*RiskBeta*(8, 1070)	
$Vp_{path.h}$	Maximum level of pathogenic Vp at harvest	Eq. (2)	log cfu/g
Risk	Risk per serving of oysters	$P_{ill} \times P_{Vp} \times P_{path}$	
r	Cases per 100,000 people	$P_{ill} \times 10^5$	person

oysters was estimated as a product of P_{ill} , P_{Vp} and P_{path}. The overall exposure model is shown in Table 4.

2.6. Data analysis

Simple statistical analysis of Kruskal-Wallis test was used to compare the climate scenarios, time-horizons and season variations due to response variables (estimated number of *V. parahaemolyticus* in a serving of oyster and the predicted annual risk per person) did not behave normally. We also performed a post-hoc comparison to compare the mean ranks of all the pairs of groups.

The risk model was developed by using a Microsoft® Excel spreadsheet (Microsoft® Excel 2019) and @Risk software (Palisade Corporation, student version 7.6). The statistical analyses were performed by using STATISTICA statistical software (version 12; StatSoft, Inc, Tulsa, USA) with a significant level of 5% of probability. The model was developed by using a Microsoft® Excel spreadsheet. The figures were then generated by OriginPro (version 2018; OriginLab Corporation, Northampton, USA).

3. Results and discussion

3.1. Evaluation of seasonal variations on the level of V. parahaemolyticus in oysters

Sixty-four scenarios with 100,000 iterations were evaluated in this study (see Appendix 2). The impact of climate change was investigated in different time-horizons and seasons. Note that the baseline values in this study were estimated based on a set of data collected at the beginning of the study and/or before intervention was applied.

Based on baseline values, the total number of this microorganism in oysters in oysters at harvest was 1.3 (95% CI: 1.3–1.3) log cfu/g, 1.5 (95% CI: 1.3–1.6) log cfu/g, 1.9 (95% CI: 1.3–1.9) log cfu/g, and 1.7 (95% CI: 1.3–1.7) log cfu/g in winter, spring, summer, and fall, respectively (Fig. 1). The estimated level of this microorganism at harvest was found in accordance with the report of Yu et al. (2013) who investigated the density of *V. parahaemolyticus* in oyster in Taiwan and found that the density of this organism in winter, spring, summer, and fall was −0.1 ± 0.3 (−1.4 – 3.5) log cfu/g, 0.5 ± 0.3 (−1.2 – 3.5) log cfu/g, 2.0 ± 0.2 (−1.2 – 4.1) log cfu/g, and 1.0 ± 0.3 (−1.4 – 4.3) log cfu/g, respectively. Hence, we considered that the model used in this study was reliable to estimate the level of this pathogen in oyster and the level of risk that may occur in Taiwan.

To ensure the safety of seafood, the International Commission on Microbiological Specifications for Foods (ICMSF, 2011) suggested the maximum limit of 4 log cfu/g of *V. parahaemolyticus* in live and raw seafood. In this study, the probability of oysters being contaminated by *V. parahaemolyticus* at levels greater than 4 log cfu/g at post-harvest was 1.5%, 2.6%, 4.6%, and 3.3% in winter, spring, summer, and fall, respectively (Fig. 2A). The probability was found to be higher with longer time-horizons and higher temperatures in climate-scenarios. Higher probability was found in RCP8.5 in 2081–2100, which was predicted to be 3.0%, 5.0%, 8.4%, and 6.2% in winter, spring, summer, and fall, respectively (Fig. 2B).

Furthermore, a Kruskal-Wallis test showed significant difference in the estimated total *V. parahaemolyticus* in oyster between seasons, $H(3, N = 64) = 53.17285$, $p < 0.001$. Multiple mean comparison with a Bonferroni adjustment between the groups showed that the estimated total of *V. parahaemolyticus* in oysters differed significantly between winter and summer ($p < 0.001$), between winter and fall ($p < 0.001$), and between spring and summer ($p < 0.001$), but not between winter and spring ($p = 0.063896$), between spring and fall ($p = 0.200644$), and between fall and summer ($p = 0.132770$). The level of *V. parahaemolyticus* in oysters in spring, summer, and fall was found to be 18%, 43%, and 29% higher than in winter. Previous studies showed that the seasonal variations affected the abundance of *V. parahaemolyticus* in oysters (Deepanjali et al., 2005; López-Hernández et al., 2015; Parveen et al., 2008), which supported the result of this study. Sobrinho et al. (2010) reported a lower density of *V. parahaemolyticus* in oyster samples harvested in the southern coastal area of Sao Paulo State, Brazil,

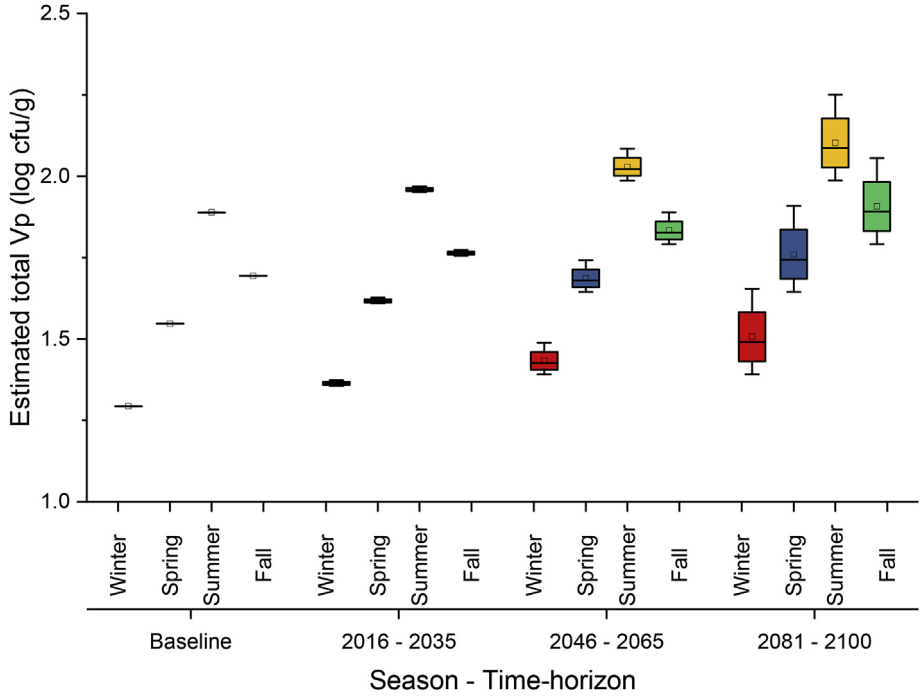

Fig. 1. Box-plot interaction between seasons and time-horizons on the estimated density of *Vibrio parahaemolyticus* in oyster at harvest. Vp = *Vibrio parahaemolyticus.*

during the winter season when mean seawater temperature and salinity were 20.1 °C and 22.3 ppt, respectively. In Taiwan, Yu et al. (2013) reported that the high density of this microorganism in water, sediment and shellfish samples was found to be associated with the decreasing salinity and high seawater temperatures in the warm season, particularly in June and September.

3.2. Evaluation of seasonal variations on the risk level of consuming raw oysters

Fig. 3 shows the interaction of seasonal variation and time-horizons on the estimated risk per serving of oysters. The US FDA (2005) estimated that ingestion of *V. parahaemolyticus* in more than 2.2×10^4 cells can cause a certain effect, such as gastroenteritis. If the total number of ingested *V. parahaemolyticus* is represented by a single cell, the US FDA (2005) estimated that the probability of illness occurring was 4.6×10^{-7}. In this study, the estimated risk of eating oysters under the baseline was estimated to be 2.2×10^{-5} (95% CI: 2.2×10^{-5} - 2.2×10^{-5}), 4.5×10^{-5} (95% CI: 2.2×10^{-5} – 4.5×10^{-5}), 9.4×10^{-5} (95% CI: 2.2×10^{-5} – 9.5×10^{-5}), and 6.2×10^{-5} (95% CI: 2.2×10^{-5} – 6.2×10^{-5}) per serving in winter, spring, summer, and fall season, respectively, higher than that suggested by the US FDA (2005). The risk per serving of oysters estimated in this study was still in the range of risk estimated in the study Huang et al. (2018) who estimated that the risk per serving of oysters in Taiwan was 8.56×10^{-5} (95% CI: 0.0–4.5×10^{-3}) per serving. However, Huang et al. (2018) did not consider the effect of seawater temperature and salinity. Besides, they also did not distinguish the risk of eating oysters based on seasonal variations. A Kruskal-Wallis test showed a significant difference in the estimation of risk per serving of oyster between the seasons in this study, $H(3, N = 64) = 52.90073$, $p < 0.001$. Multiple mean comparison with a Bonferroni adjustment between the groups showed that the estimated risk of *V. parahaemolyticus* infection from eating our study's oysters differed significantly between winter and spring ($p = 0.048446$), between winter and summer ($p < 0.001$), between winter and fall ($p < 0.001$), and between spring and summer ($p < 0.001$), but not between spring and fall ($p = 0.277023$) and between summer and fall ($p = 0.114124$). Based on this analysis, it was

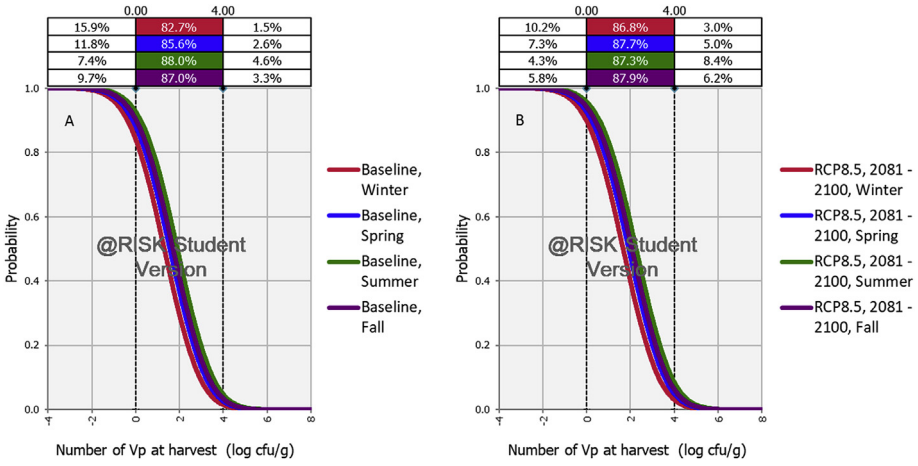

Fig. 2. Cumulative distribution of total *Vibrio parahaemolyticus* in oyster at harvest in baseline (A) and RCP8.5 in 2086–2100 (B).Vp = *Vibrio parahaemolyticus.*

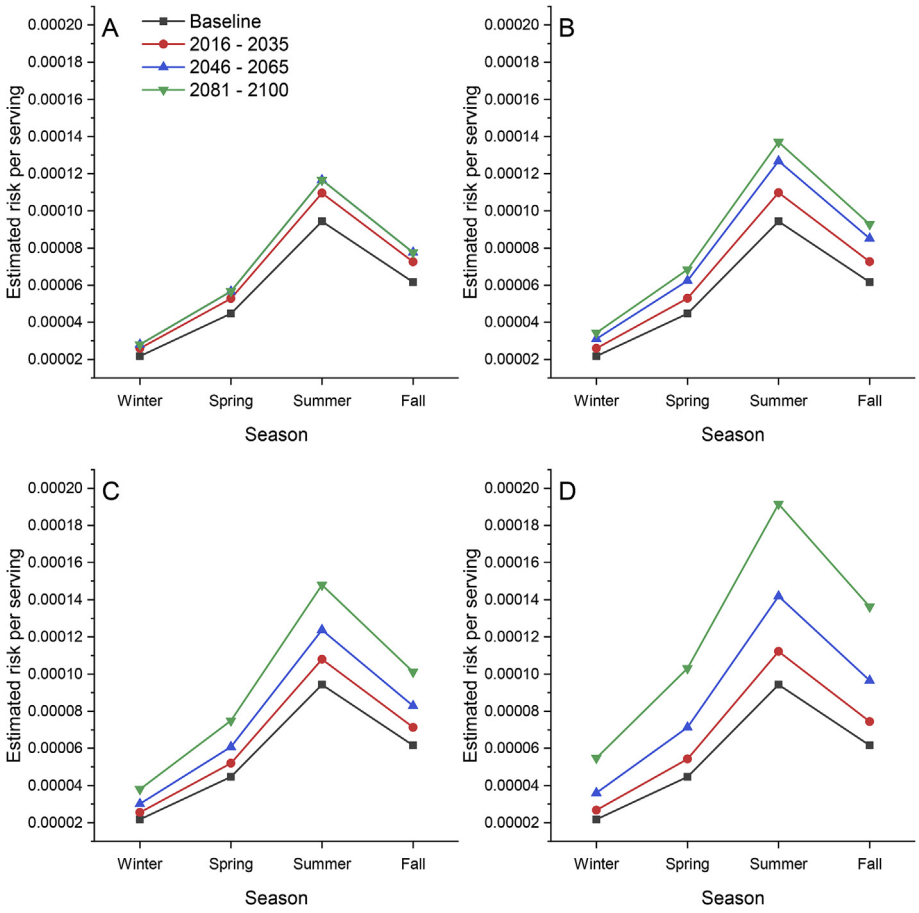

Fig. 3. Interaction between season-time horizon climate scenarios on the estimated risk per serving of oysters in RCP2.6 (A), RCP4.5 (B), RCP6.0 (C), and RCP8.5 (D).

found that the risk per serving of oysters in summer was found 0.5, 1.0, and 3.1 times higher than that in fall, spring, and winter, respectively.

3.3. Evaluation of climate scenarios at different time horizons

A Kruskal-Wallis test showed that the estimated risk per serving of oysters between the time-horizons did not differ significantly, $H(3, N = 64) = 7.357098$, $p = 0.0613$. The risk per serving in baseline was intensified to 0.18, 0.38, and 0.64 times in 2016–2035, 2046–2065, and 2081–2100, respectively. Nevertheless, the highest risk per serving that probably occurs was predicted in 2081–2100 with RCP8.5, which was estimated to be 5.5×10^{-5} (95% CI: 2.2×10^{-5} – 5.6×10^{-5}), 1.0×10^{-4} (95% CI: 2.2×10^{-5} – 1.0×10^{-4}), 1.9×10^{-4} (95% CI: 2.2×10^{-5} – 1.9×10^{-4}), and 1.4×10^{-4} (95% CI: 2.2×10^{-5} – 1.4×10^{-4}) in winter, spring, summer, and fall, respectively.

Furthermore, another Kruskal-Wallis test showed that the climate scenarios failed to be a significant factor for the estimated risk per serving of oysters in this study, $H(3, N = 64) = 5.749015$, $p = 0.2187$. The result in this study was in contrast to Ortiz-Jiménez (2018) who found that the risk per serving of raw oysters differed significantly between the climate scenarios. The risk per serving of raw oyster in Boca de Camichín, Mexico would increase to 1.12 times from 2020 to 2100, presuming that no mitigation interventions were performed. In the study of Ortiz-Jiménez (2018), the risk was estimated based on conditions such as time-temperature exposure, raw oyster treatment, and initial bacterial load, that were generally different from those in the present study.

Although climate change does not significantly increase the risk of consuming oysters in Taiwan as simulated in this study, it could intensify the risk in the baseline up to 23%, 35%, 37%, and 65% based on RCP2.6, RCP4.5, RCP6.0, and RCP8.5, respectively. Referring to the assessment performed by the IPCC group, the worst case in RCP8.5 may occur because there was no agreement among the members to decrease the greenhouse effect (IPCC, 2014). This indicated that mitigation strategies to reduce and/or control the presence or growth of pathogenic bacteria in oysters are necessary.

3.4. Evaluation of mitigation strategies

Our interest was in the evaluation of the mitigation strategies against the baseline. According to the International Commission on Microbiological Specifications for Foods (ICMSF), the maximum density of *V. parahaemolyticus* in live and raw seafood should be lower than 4 log cfu/g (ICMSF, 2011). In this study, we found that immediate refrigeration, depuration, or freezing treatment still allows this pathogen to exceed the limit of 4 log cfu/g before eating (Fig. 4A), which indicated that these treatments were considered ineffective in preventing the proliferation of *V. parahaemolyticus*. This case could be true because these treatments could only allow the reduction of *V. parahaemolyticus* density in oysters up to 2 orders of magnitude, which still allows the density of this microorganism to be higher than the maximum limit.

Several researchers have demonstrated that this pathogen could not grow or at least remains inactivated at low temperature (Cook & Ruple, 1989; Gooch, Depaola, Bowers, & Marshall, 2002; Limthammahisorn et al., 2009). The recent investigation of Jones et al. (2017) suggests that the oysters should be refrigerated immediately at post-harvest to inhibit the growth of *V. parahaemolyticus* in oysters; they found that the oyster samples without refrigeration had a higher density of *V. parahaemolyticus* than that in the refrigerated samples. Additionally, they also observed a significant difference in the density of this

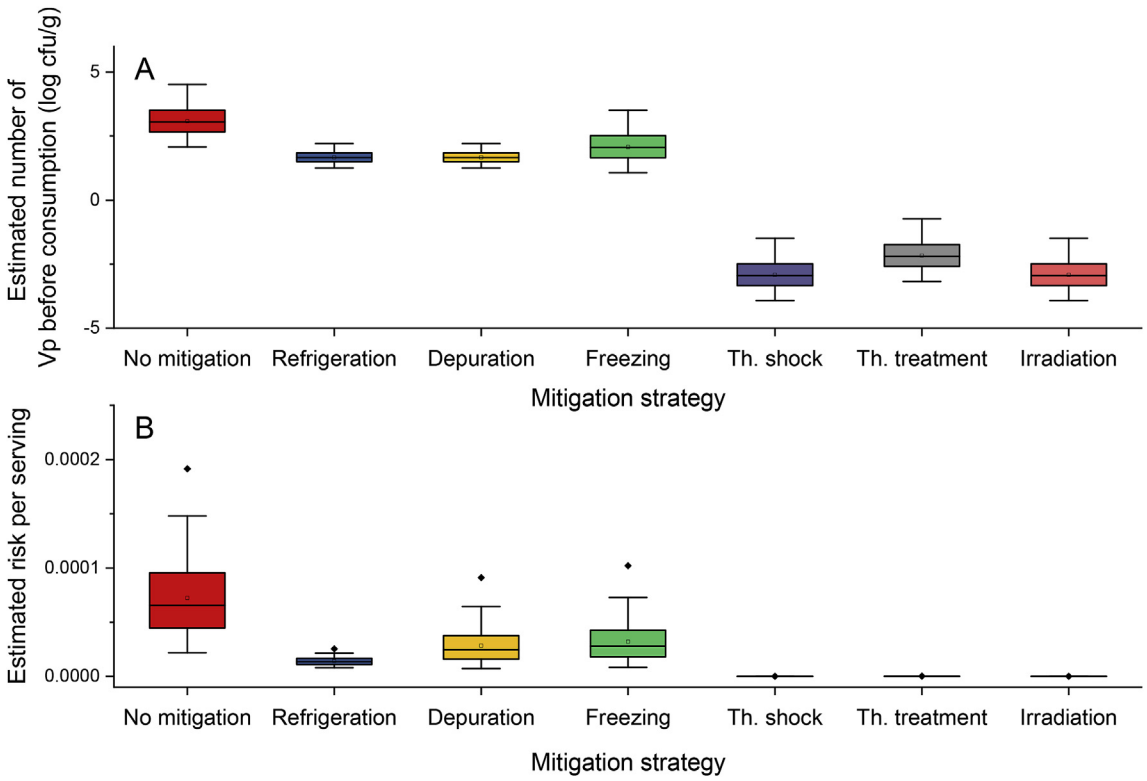

Fig. 4. The effect of mitigation strategies on the estimated level of *Vibrio parahaemolyticus* before eating (A) and the risk of getting ill from a serving of oysters (B).

microorganism before and after exposing the oysters at ambient temperature for more than 5 h. Furthermore, Ramos et al. (2012) reported that depuration treatment could reduce the number of *V. parahaemolyticus* in oysters up to 2.4 orders of magnitude after 48 h of treatment in seawater with UV light. A high effect of depuration (3.1 orders of magnitude) could be obtained by immersing the oysters in seawater with UV light plus a certain amount of chlorine. Moreover, freezing treatment followed by frozen storage could inactivate *V. parahaemolyticus* in oysters (Liu et al., 2009; Muntada-Garriga et al., 1995). Liu et al. (2009) reported that this pathogen declined by 2.45 log MPN/g, 1.71 log MPN/g, and 1.45 log MPN/g after 1 month of storage and by 4.55 log MPN/g, 4.13 log MPN/g, and 2.53 log MPN/g after 6 months of storage at −10 °C, −20 °C and - 30 °C, respectively. In another study of Muntada-Garriga et al. (1995) revealed that these bacteria were inactivated from 7 log cfu/g to a none detectable level after 27 d and 28 d at −18 °C and −24 °C, respectively.

Obviously, mild thermal treatment, thermal shock, and irradiation simulated in this study were found capable of reducing the population of this microorganism below the allowed maximum limit suggested by the ICMSF (2011). Previous studies have shown that mild thermal treatment or thermal shock does not impart a noticeable cooked appearance or taste to the oysters (Andrews, DeBlanc, Veal, & Park, 2003, 2000). The use of such kind of method has been implemented commercially for reducing or eliminating Vibrios in oysters in the United States (Hesselman, Motes, & Lewis, 1999). Moreover, the use of irradiation treatment to eliminate the pathogenic bacteria in food has also been approved in the United States (US FDA, 2018) and European countries (EC [European Commission], 1999). Irradiation treatment is quite efficient in eliminating the population of *V. parahaemolyticus* in oysters. Besides, this method keeps the oysters alive and does not affect the oyster's sensory reactions. However, a challenge may be faced by the shellfish harvesters due to the limited amount of irradiation facilities at their sites.

Fig. 4B shows the effect of mitigation strategies on the estimated risk per serving of oysters. This figure demonstrates that each mitigation strategy has a different effect on the risk per serving of oysters. A Kruskal-Wallis test showed a significant difference on the estimated risk per serving of oyster between the mitigation strategies, H (6, N = 448) = 391.5908, $p < 0.001$. The mean rank of oysters without mitigation (M Rank = 397.81) was higher than with refrigeration (M Rank = 251.28), depuration (M Rank = 310.66), freezing (M Rank = 322.25), thermal treatment (M Rank = 153.11), thermal shock (M Rank = 89.06), and irradiation (M Rank = 47.33). Multiple mean comparisons with a Bonferroni adjustment showed that the risk per serving in all mitigation strategies differed significantly compared to oysters without mitigation intervention. This study found that immediate refrigeration, depuration, and freezing treatment could reduce the risk per serving in oysters up to 64–87%, 52–67%, and 47–63%, respectively. Mild thermal treatment, thermal shock and irradiation could reduce the risk up to 100%.

4. Conclusion

This study assessed the risk of *V. parahaemolyticus* infection associated with raw oyster consumption under the seasonal variations, time horizons, and climate scenarios in Taiwan. As simulated in this study, these factors did affect the risk per serving of oyster. The level of *V. parahaemolyticus* in raw oyster in summer was predicted 11%, 21%, and 43% higher than that in fall, spring and winter, respectively. Consequently, the risk in summer was estimated to be 0.5, 1.0, and 3.1 times higher than that in fall, spring and winter, respectively. In addition, risk was predicted to increase gradually as the global climate worsens and under time horizons. The risk in the baseline in this study was found to intensify by 23%, 35%, 37%, and 65% by RCP2.6, RCP4.5, RCP6.0, and RCP8.5, respectively, and it to 0.18, 0.38, and 0.64 times in 2016–2035, 2046–2065, and 2081–2100, respectively. Nevertheless, mitigation strategies simulated in this study could significantly reduce the risk in eating oysters. Immediate refrigeration, depuration, and freezing treatment could reduce the risk compared to oyster with no mitigation by 64–87%, 52–67%, and 47–63%, respectively. In this

study, we recommend the implementation of mild thermal treatment, thermal shock and irradiation treatment by oyster processors in Taiwan as it could reduce the risk by up to 100%. Reducing and or controlling the risk of raw oysters will prevent foodborne outbreaks. Finally, the findings obtained from this study may help the food safety authority and food manager in food industry in their decision-making process.

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Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2019.03.020.

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